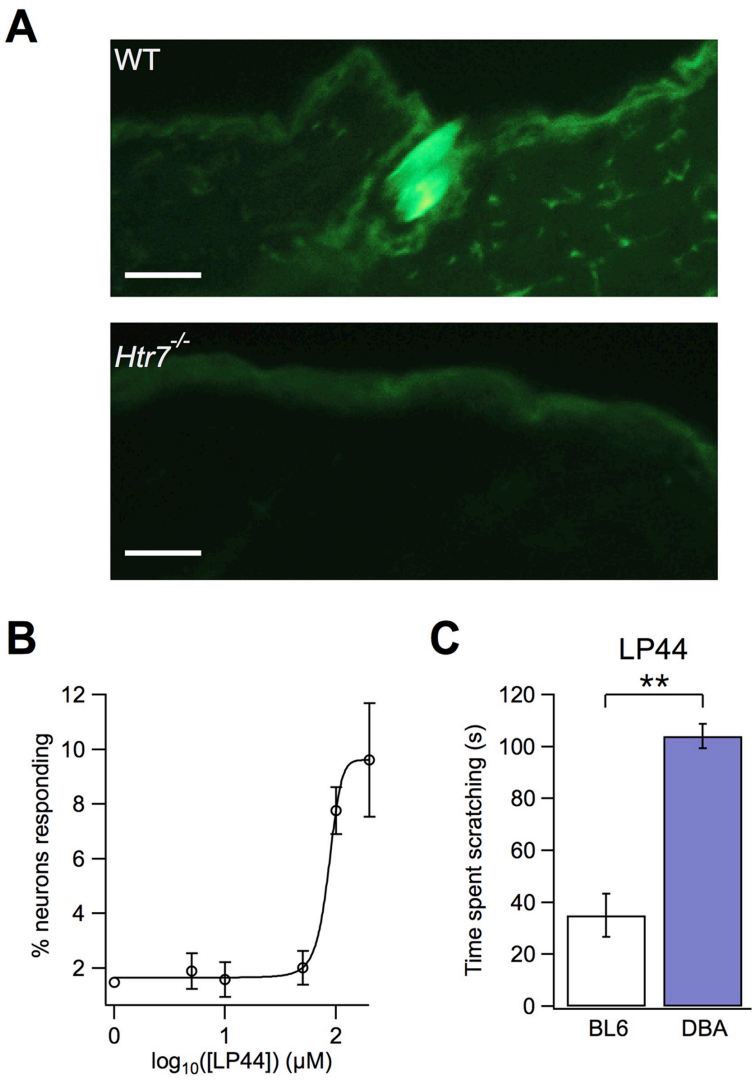
A
WT
Htr7-/-
B
% neurons responding
log10([LP44]) (μM)
C
LP44
**
Time spent scratching (s)
BL6
DBA

**Figure S1**

## SUPPLEMENTAL FIGURE LEGENDS

**Figure S1, Related to Figures 2 and 4. LP44 activates a subset of sensory neurons that express HTR7.**

(A) Validation of HTR7 antibody. Immunohistochemistry of hairy skin sections with an antibody against HTR7 shows neuronal staining in WT (top) but not in *Htr7*$^{-/-}$ (bottom). Scale bar, 50 μm. (B) Dose-response curve of LP44 on primary DRG culture assayed by calcium imaging, $n$ = 50-100 cells/concentration. (C) LP44 evokes differential itch response between BL6 and DBA. Student's t-test, $^{**}p < 0.01$, $n$ = 7/genotype. Error bars represent SEM.

## SUPPLEMENTAL TABLES

### Table S1, Related to Figure 1. Top candidate itch genes.

List of candidate genes displaying strong correlation between DRG gene expression and CQ-evoked itch behavior ($|r| > 0.55$).

| Positive genes | | | | Negative genes | |
|---|---|---|---|---|---|
| *r* | Gene symbol | *r* | Gene symbol | *r* | Gene symbol |
| 0.704 | *Ppig* | 0.582 | *Kcnc3* | -0.670 | *Gm14399* |
| 0.680 | *Gm15535* | 0.582 | *Flt3* | -0.634 | *4931406C07Rik* |
| 0.674 | *Spsb4* | 0.581 | *Endod1* | -0.630 | *Rqcd1* |
| 0.666 | *Arl4c* | 0.580 | *Fam184b* | -0.617 | *BC030867* |
| 0.650 | *Ppargc1a* | 0.579 | *Faim2* | -0.612 | *Gm14326* |
| 0.649 | *Htr7* | 0.578 | *Agl* | -0.610 | *Atp1b3* |
| 0.645 | *Slc35d3* | 0.578 | *Scn8a* | -0.604 | *Tmem176a* |
| 0.638 | *Inhbb* | 0.577 | *Mtap2* | -0.599 | *BC048355* |
| 0.638 | *Phkb* | 0.577 | *Cbln2* | -0.598 | *2210404O09Rik* |
| 0.632 | *Unc80* | 0.576 | *Ano1* | -0.593 | *Ada* |
| 0.629 | *Zfp940* | 0.573 | *1700040D17Rik* | -0.588 | *Med22* |
| 0.626 | *2610528B01Rik* | 0.572 | *Gm10676* | -0.583 | *Tmem176b* |
| 0.625 | *Tmem47* | 0.572 | *Shh* | -0.581 | *Pla2g16* |
| 0.617 | *Gm17638* | 0.571 | *2510049J12Rik* | -0.580 | *Gm14325* |
| 0.616 | *Htr1d* | 0.570 | *Phospho1* | -0.578 | *Sh3gl1* |
| 0.615 | *Hapln4* | 0.569 | *Rasa1* | -0.576 | *Mrps6* |
| 0.614 | *Myh14* | 0.568 | *Atp2b2* | -0.575 | *Gm14305* |
| 0.613 | *Onecut1* | 0.565 | *D630023F18Rik* | -0.572 | *Cdkn1a* |
| 0.611 | *Cabp7* | 0.564 | *Kcnq4* | -0.572 | *Api5* |
| 0.611 | *Cnnm1* | 0.562 | *Ppp1r12b* | -0.569 | *Sertad1* |
| 0.610 | *Cpsf6* | 0.559 | *Cables2* | -0.569 | *Casp1* |
| 0.607 | *Gm17111* | 0.558 | *Ogdhl* | -0.568 | *Gm6543* |
| 0.607 | *Esrrg* | 0.558 | *Cnnm4* | -0.568 | *Wtap* |
| 0.604 | *Wipf2* | 0.557 | *Kcnh7* | -0.565 | *0610040B10Rik* |
| 0.604 | *Cntnap2* | 0.556 | *Epb4.1l1* | -0.565 | *Rnh1* |
| 0.602 | *Rtbdn* | 0.555 | *Papd5* | -0.564 | *Rxrg* |
| 0.602 | *Sh3rf3* | 0.555 | *Scrt2* | -0.564 | *Bcl10* |
| 0.601 | *1700007I08Rik* | 0.554 | *Ube3b* | -0.563 | *Cd9* |
| 0.601 | *Dynll2* | 0.553 | *Gm17443* | -0.563 | *Setd6* |
| 0.596 | *Pcdh8* | 0.553 | *Cds1* | -0.561 | *Ctnna1* |
| 0.595 | *Kcnc1* | 0.553 | *Scrt1* | -0.559 | *Trp53i13* |
| 0.591 | *Heatr5a* | 0.551 | *A930033H14Rik* | -0.558 | *Insc* |
| 0.591 | *9630001P10Rik* | 0.551 | *Hhatl* | -0.558 | *Dok1* |
| 0.590 | *Chgb* | 0.550 | *Myrip* | -0.557 | *Zfp119b* |
| 0.589 | *Clstn2* | 0.550 | *Gm17456* | -0.556 | *Gm7325* |
| 0.585 | *Strbp* | | | -0.556 | *Hmgcl* |
| 0.583 | *Bcl2* | | | -0.552 | *Tssc4* |

**Table S2, Related to Figure 1. Gene ontology (GO) enrichment of top candidate itch genes.**

List of GO terms that are enriched in top candidate genes displaying strong correlation between DRG gene expression and CQ-evoked itch behavior ($r$ > 0.55).

| GO ID | Term | *p*-value |
|---|---|---|
| GO:0022843 | voltage-gated cation channel activity | 0.00011 |
| GO:0005251 | delayed rectifier potassium channel activity | 0.00019 |
| GO:0005249 | voltage-gated potassium channel activity | 0.0002 |
| GO:0005516 | calmodulin binding | 0.00027 |
| GO:0015267 | channel activity | 0.00035 |
| GO:0022803 | passive transmembrane transporter activity | 0.00035 |
| GO:0005244 | voltage-gated ion channel activity | 0.00041 |
| GO:0022832 | voltage-gated channel activity | 0.00041 |
| GO:0022836 | gated channel activity | 0.00046 |
| GO:0022839 | ion gated channel activity | 0.00046 |
| GO:0005267 | potassium channel activity | 0.00075 |
| GO:0004993 | serotonin receptor activity | 0.00111 |
| GO:0015079 | potassium ion transmembrane transporter activity | 0.00119 |
| GO:0046873 | metal ion transmembrane transporter activity | 0.00144 |
| GO:0005216 | ion channel activity | 0.00174 |
| GO:0022838 | substrate-specific channel activity | 0.00182 |
| GO:0005261 | cation channel activity | 0.00206 |
| GO:0001948 | glycoprotein binding | 0.00276 |
| GO:0015077 | monovalent inorganic cation transmembr... | 0.00292 |
| GO:0022890 | inorganic cation transmembrane transport... | 0.0032 |

## SUPPLEMENTAL EXPERIMENTAL PROCEDURES

### RNA extraction and library preparation

DRG neurons from one animal of each BXD progeny strain (see Figure 1B, bottom, for strains used) were removed and homogenized in Trizol (Life Technologies). Total RNA was extracted according to the manufacturer's protocol and genomic DNA was removed with Turbo DNase I (Life Technologies). Libraries were prepared using TruSeq RNA Sample Prep kit v2 (Illumina) and sequenced on Illumina HiSeq 2000 machines with 50bp single-end reads to obtain 30-40 million reads per sample.

### RNA-seq analysis

To map reads from DRG RNA-seq libraries from BXD mice, we first generated a DBA/2J reference genome by amending the C57BL/6J genome (www.ensembl.org, release NCBIM37) with DBA/2J SNPs and indels (http://www.sanger.ac.uk, release 'REL-1211-SNPs_Indels'), using only those with a genotype quality Phred score of 99. We then mapped each RNA-seq read set to this DBA/2J reference and, separately, to the C57BL/6J reference, using Tophat (Trapnell et al., 2009) in each case. We retained for analysis only those reads in each mapping run that mapped uniquely with no mismatches. For a given read that met this criterion for each of the two references, we eliminated it from further analysis if the position to which it mapped was not orthologous between the two. HTSeq (Anders et al., 2015) was used to sum the total read counts per gene. Read counts were normalized with the EDASeq package in R

(Risso et al., 2011) using the upper-quartile method. Gene ontology enrichment analysis was performed using the topGO package in R (Alexa et al., 2006).

**Histology**

For immunohistochemistry, caudal back skin tissue was harvested from mice and flash frozen in OCT (Tissue-Tek). Tissue was cryosectioned at 14 - 20 µm and mounted on glass coverslips for staining. Histology was carried out as previously described (Gerhold et al., 2013). Antibodies: sheep anti-TRPA1 100 µg/ml (Abcam), rabbit anti-HTR7 1:5000 (Abcam), Alexa 488 goat anti-rabbit 1:5000 (Life Technologies), and Alexa 568 donkey anti-sheep 1:5000 (Life Technologies). Skin sections were counterstained with DAPI (Life Technologies). For *in situ* hybridization, DRG were harvested and sectioned as described for immunohistochemistry. *Htr7* probes (Panomics) were used following the Quantigene protocol (Panomics). Images were taken using a Zeiss LSM710 confocal microscope at the Biological Imaging Facility, UC Berkeley.

**Cell culture**

Preparation of neurons and ratiometric $Ca^{2+}$ imaging were carried out as previously described (Wilson et al., 2011). Briefly, neurons from sensory ganglia were dissected and incubated for 10 min in 1.4 mg $ml^{-1}$ Collagenase P (Roche) in Hanks calcium-free balanced salt solution, followed by incubation in 0.25% standard trypsin (vol/vol) STV versene-EDTA solution for 3 min with gentle agitation. Cells were then triturated , plated onto glass coverslips and used within

20 h. (media: MEM Eagle's with Earle's BSS medium, supplemented with 10% horse serum (vol/vol), MEM vitamins, penicillin/streptomycin and L-glutamine). For retrograde labeling of cutaneous afferent neurons, cholera toxin subunit B, Alexa Fluor 594 conjugate (Life Technologies) was intradermally injected (4mg/ml in PBS, 10 µl), and after 24 h, sensory neurons were dissected and cultured as described above. HEK293 cells (ATCC) were transfected with 500 ng of GFP tagged human HTR7 plasmid (Origene) either alone or with 50 ng of human TRPA1 using Lipofectamine 2000 (Life Technologies) per the manufacturer's instructions. Cells were plated on glass coverslips and used within 24 h for calcium imaging and whole cell recordings. Mouse primary keratinocytes (Yale Dermatology Cell Culture Facility) were plated on glass coverslips and used within 24 h for calcium imaging. All media and cell culture supplements were from the UCSF Cell Culture Facility.

**Electrophysiology**

Whole cell recordings in transfected cells were performed using the Port-a-patch system (Brueggemann et al., 2004) (Nanion Technologies). All solutions used were provided by the manufacturer. Briefly, whole cell configuration was achieved with a high calcium external solution (in mM: 80 NaCl, 3 KCl, 10 $MgCl_2$, 35 $CaCl_2$, 10 HEPES, pH 7.4) which was replaced with a conventional external solution (in mM: 140 NaCl, 4 KCl, 1 $MgCl_2$, 2 $CaCl_2$, 5 d-Glucose monohydrate, 10 HEPES, pH 7.4) prior to recording. The internal solution consisted of (in mM) 50 KCl, 10 NaCl, 60 KF, 20 EGTA, 10 HEPES, pH 7.2. Cells were held at -80 mV

and ramped every second from -100 mV to 100 mV over 300 ms. Inward currents were determined at -100 mV and plotted as a function of time. Sensory neuron recordings were collected at 5 kHz and filtered at 2 kHz using an Axopatch 200B and PClamp software. Electrode resistances were 2–6 MΩ. Stimulation protocol: 10 ms step to −80 mV, 150 ms ramp from −80 mV to +80 mV. Current clamp internal solution (in mM): 140 KCl, 5 EGTA, and 10 HEPES (pH 7.4 with KOH). Series resistance of all cells were <30 MΩ. Cells were perfused with external solution containing either 100 μM LP-44 (Sigma Aldrich) or 100 μM AITC (Sigma Aldrich). Cells were defined to be responsive to LP-44 if there was at least a 190% increase in current (chosen based on an average increase of 184% in the HTR7-TRPA1 co-transfected cells).

**Calcium imaging**

$Ca^{2+}$ imaging experiments were carried out as previously described (Wilson et al., 2011). Cells were loaded for 30 min at room temperature with Fura-2AM, 10 μM for neuronal culture, and 2 μM for keratinocytes and HEK293 cells, supplemented with 0.01% Pluronic F-127 (wt/vol, Life Technologies), in a physiological Ringer's solution containing (in mM) 140 NaCl, 5 KCl, 10 HEPES, 2 $CaCl_2$, 2 $MgCl_2$ and 10 D-(+)-glucose, pH 7.4. All chemicals were purchased from Sigma. Acquired images were displayed as the ratio of 340 nm to 380 nm. Cells were identified as neurons by eliciting depolarization with high potassium solution (75 mM) at the end of each experiment. Neurons were deemed to be sensitive to an agonist if the peak response was ≥10% above baseline. Image analysis and

statistics were performed using custom routines in Igor Pro (WaveMetrics). All graphs displaying Fura-2 ratios were normalized to the baseline ratio F340/F380 = (Ratio)/(Ratio t = 0).

**PCR**

cDNA was synthesized using SuperScript III reverse transcriptase (Life Technologies). Samples were diluted 1:10 and used as template for PCR experiments. The following primer pairs were used: mouse *Htr7* (forward, 5'-GCCATCTCCGCTCTCTCATC-3'; reverse, 5'-CCATAGTTGATCTGCTCCCCG-3'), mouse *Gapdh* (forward, 5'-CCGTAGACAAAATGGTGAAGGT-3'; reverse, 5'-GGGCTAAGCAGTTGGTGGT-3'), human *Htr7* (forward, 5'-CCCAGAGCAGTGTTTGTTTCA-3'; reverse, 5'-AGACCCTTCAGAGCACGAGA-3'), human *Gapdh* (forward, 5'-CCACTCCTCCACCTTTGAC-3'; reverse, 5'-ACCCTGTTGCTGTAGCC-3').

**Behavioral studies**

*Htr7*$^{-/-}$ mice were obtained from Jackson Laboratory (Hedlund et al., 2003), *Trpv1*$^{-/-}$ and *Trpa1*$^{-/-}$ mice were described previously (Bautista et al., 2006; Caterina et al., 2000), and *K14-Cre;Trpa1*$^{fl/fl}$ mice were provided by Cheryl Stucky (Zappia et al., in preparation). Mice (20–35 g) were housed in 12 h light-dark cycle at 21°C. Itch behavioral measurements were performed as previously described (Wilson et al., 2011). Compounds injected: 2 mM LP44 (Santa Cruz), 100 µM or 1 mM 5-HT (Sigma), 40 mM chloroquine (Sigma), 3.5 mM BAM8-22 (Tocris), 4 mM

compound 48/80 (Sigma), 15 nM TSLP (R&D Systems), 100 μM sertraline (Tocris), and 27 mM histamine (Tocris) in PBS. For all behavioral experiments, pruritogens were injected using both the neck model (50 μl), and the cheek model (20 μl) of itch, as previously described (Wilson et al., 2011); all differences observed between wild type and knockout animals did not vary by injection site. For the neuronal ablation experiment, resiniferatoxin (1 μg/ml in 0.05% ascorbic acid and 7 % tween 80) was injected to the cheek two days prior to pruritogen injection. For AITC behavior, 5 μl 10% AITC (Sigma) in mineral oil was applied to the right hind paw. Radiant heat paw withdrawal latencies, before and after injection with LP44 or 5-HT were performed as previously described (Tsunozaki et al., 2013). All mice were acclimated behavioral chambers on 2 subsequent days prior to treatment. Mice were injected with 20 μl LP44 (2 mM) or 5-HT (10 μM) into the hind paw, and their paw withdrawal latencies were measured 15 min pre- and 15 min and 30 min post-injection. Behavioral scoring was performed while blind to experimental condition and mouse genotype. All experiments were performed under the policies and recommendations of the International Association for the Study of Pain and approved by the University of California, Berkeley Animal Care and Use Committee.

**Vitamin D model of Atopic Dermatitis**

Mice were singly housed and cheek hair was shaved one week prior to the start of the assay. 200 μM (in 20 μl EtOH) MC903 (R&D Systems) was applied to the cheek once per day, for 7 days. Behavior was recorded for 30 minutes on days 3,

5, 7 (ongoing treatment) and days 8, 10 and 12 (post-treatment) and the time spent scratching was quantified. Character of lesion scoring (COL) was adapted from (Hampton et al., 2012; Liu et al., 2013; Yun et al., 2011). Lesion severity was scored for redness (erythema), dryness (xerosis), and scabbing (excoriation) based on a 0-3 scale as follows: 0 = none, 1 = mild, 2 = moderate, 3 = severe. Behavioral and lesion scoring was performed while blind to genotype, treatment, and treatment duration.

**Serotonin measurements**

Cheek skin was homogenized in 0.2 N $HClO_4$ (10 µl/mg of tissue) and neutralized with equal volume of 1 M borate buffer (pH 9.5). 5-HT levels were measured using the serotonin ELISA kit (Beckman Coulter).

**Statistical analysis**

Values are reported as the mean ± s.e.m. For comparison between two groups, Student's *t*-test was used. For single-point comparison between >2 groups, a one-way ANOVA followed by a Tukey-Kramer *post hoc* test was used. For the time course comparison between >2 groups, ANOVA for multivariate linear models was used. Significance was labeled as: ns, not significant, $p \geq 0.05$; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

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